INVESTIGATIONS OF THE DETERMINATION OF CALCIUM IN THE PRESENCE OF MAGNESIUM

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INVESTIGATIONS OF THE DETERMINATION OF CALCIUM IN THE PRESENCE OF MAGNESIUM

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SUMMARY

Classical procedures for the determination of calcium are tedious and time consuming. Earlier methods for the complexometric determination of calcium offer much improvement, but serious problems result when magnesium is present. The introduction of EGTA helped the situation somewhat. Even though the stability constants for calcium-EGTA and magnesium-EGTA are not different enough to allow a visual titration, a photometric determination of calcium in the presence of magnesium should be possible. Thus, it was felt that additional work in this area should be done.

In order to have a theoretical basis upon which to make this investigation, the concepts of conditional stability and photometric indication were reviewed. Using these concepts, it was determined that a slope-indication method offered the highest probability of finding a system which would allow the titration of calcium without the necessity of masking magnesium. Since PAR forms highly absorbing, water-soluble complexes with many metals, it was felt that such complexes could be used as slope-indicators.

The conditional stability constants of many metal-EGTA chelonates in the presence of PAR were calculated, and the data from these results were examined to determine which metal-PAR complex should work best as a slope-indicator.

The experimental work showed that copper-PAR was the only PAR complex investigated which will work well in this context, and that small amounts of magnesium can be tolerated when using such an indicator.

CHAPTER I

INTRODUCTION

Methods of photometric analysis are based on the Lambert-Beer [1] Law which gives the linear relationship between the absorbance of a beam of light passing through a solution and the concentration of the absorbing species. The relationship is expressed mathematically as

$$A = \text{Log } \frac{1}{T} = \text{Log } \frac{P}{P_o} = abc \qquad (1)$$

where

A = the absorbance

T = the transmittance

P = the radiant power of the beam of light transmitted by the sample

P_o = the radiant power of the beam of light incident on the sample

a = the absorptivity, which is a measure of the ability of a species to absorb light of a certain wavelength

b = the length of the light path through the absorbing medium

c = the concentration of the absorbing species

If a reaction takes place in a reasonably fast and

predictable manner, and if at least one component involved in the reaction absorbs light, then the possibility exists of performing a photometric titration. By plotting absorbance (ordinate) vs. volume of titrant (abscissa), one obtains the titration curve. From a knowledge of which components absorb light and to what degree (absorptivity), the shape of the titration curve can be predicted.

For the case of interest here, namely a complexation titration, the general titration equation may be presented as

$$A + B \leftrightharpoons C \qquad (2)$$

In the case of titrating "A" with titrant "B", one is able to predict the theoretical shape of a titration curve with the assumption that one or more the the components absorb light of the chosen wave length.

Figure 1 presents the titration curves for all possible combinations of light absorbing species in Reaction Equation 2. Figure 1-III and 1-V present the case where no break occurs in the curve because one of the reactants and the product have the same absorptivity at the monitoring wavelength. However, this is rarely a problem in practice since two compounds hardly ever absorb equally at all wavelengths available for conducting the titration.

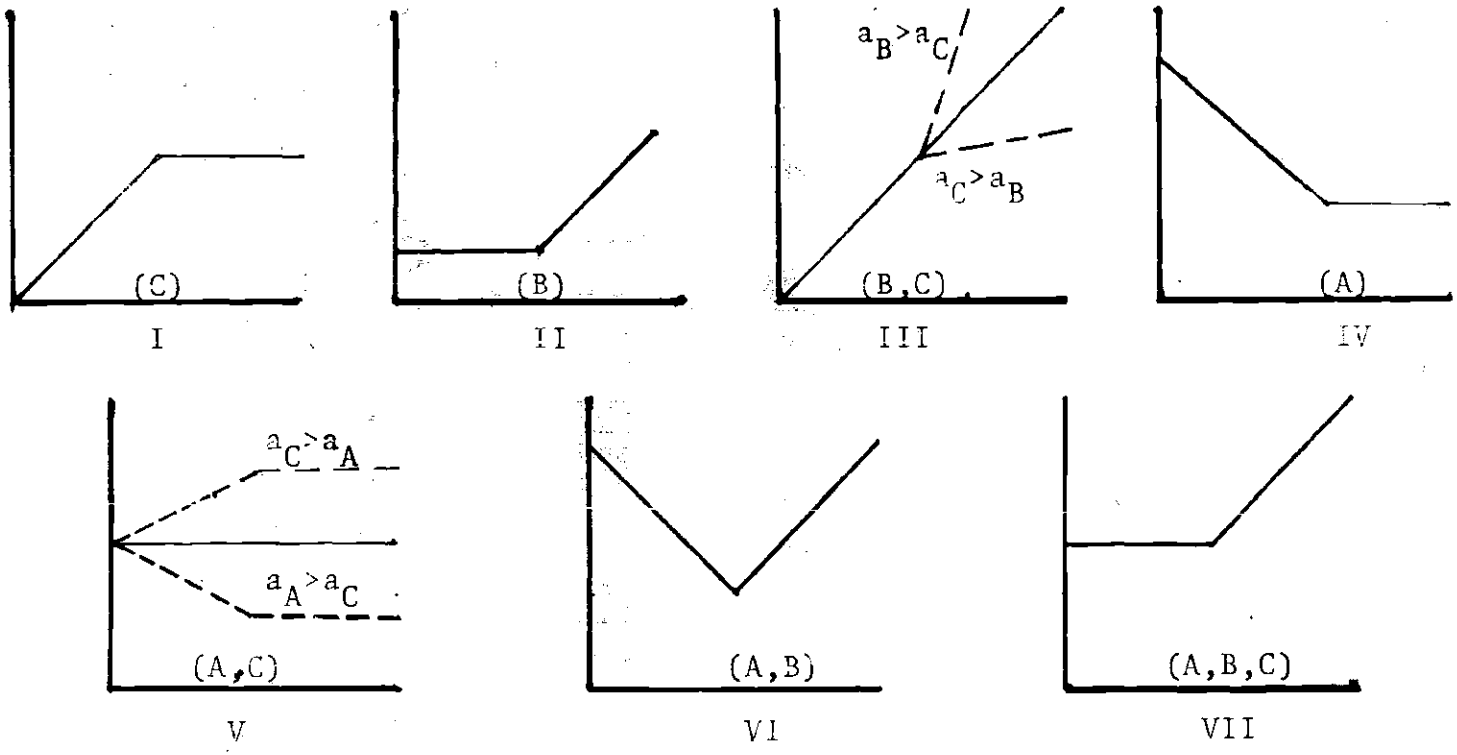

Figure 1. Titration Curves for all Possible Combinations of Light Absorbing Species in Reaction Equation 2. (Letters in parenthesis indicate absorbing species.)

Photometric End Point

If a titration is monitored photometrically, the break which appears in the titration curve is taken as the photometric end point. In order for a photometric titration to be useful, the break in the titration curve must occur at or very near the equivalence point.

Depending on the shape of the titration curve and the underlying titration reaction, three types of photometric indication may be differentiated, namely self-indication, step-indication, and slope-indication. These will briefly be discussed below. A paper by Flashka and Sawyer [2] contains a more extensive development of the concepts.

Self-Indication

When one of the components participating in the titration equilibrium absorbs at the monitoring wavelength or can be brought to do so by transfer into an appropriate form, self indication of the photometric end point is possible. All of the titration curves shown in Figure 1 pertain to self indication.

Perhaps the best known example of self indication is the titration of iron (II) with permanganate, which is commonly conducted visually. If the titration is followed photometrically, then a titration curve such as that shown in Figure 1, Curve II, will result.

Another example is the complexometric titration of copper with EDTA which may be conducted in two ways. In the

first method the copper is present in solution as the weakly absorbing aquo complex, and upon addition of EDTA is transferred to the more strongly absorbing EDTA complex. When the titration is monitored at the wavelength at which copper-EDTA absorbs, one obtains a titration curve such as that shown in Figure 1, Curve 1.

In the second method of titration the copper is present in solution as the strongly absorbing copper-ammine complex, and upon addition of EDTA is transferred to the weaker absorbing EDTA complex. When the titration is monitored at the wavelength at which copper-ammine absorbs, one obtains a titration curve such as that shown in Figure 1, Curve IV. The titration of copper in the presence of an excess of ammonia is preferred because the absorptivity of the copper-ammine complex is larger than that of the aquo of EDTA complex, thus yielding curves with larger slope differences.

Step-Indication

Step-indication, having two types of curves, is best explained with an actual example. The first type, (Figure 2, Curve I), is commonly known as a "step up" curve. The second type (Figure 2, Curve II), is known as a "step down" curve.

The photometric titration of magnesium with EDTA using a small amount of Erio T as indicator will give a step up curve if the uncomplexed Erio T is monitored, or a step down curve if the magnesium-Erio T complex is monitored. In both

titrations, the second break in the titration curve corresponds to the end point for the total magnesium in solution. The first break point indicates the point where all the magnesium not complexed with the Erio T has been titrated. The sloping portion of the titration curve corresponds to the self-indicating titration of the magnesium-Erio T complex.

In order to obtain a titration curve using step-indication, the amount of indicator added must be less than the equivalent amount of metal to be determined. If the amount of indicator (Erio T) is equivalent or in excess to the species titrated (magnesium) then step indication no longer prevails, but a self-indicating system is created. The Erio T now has the exact same function with regard to the magnesium as did the ammonia in the copper titration; the Erio T now should no longer be called the "indicator."

Slope-Indication

Slope-indication is also best explained with practical examples of which the first is the complexometric titration of bismuth with EDTA as reported by Underwood [3]. A curve such as that shown in Figure 2, Curve III, results when using copper as the slope indicator. Bismuth forms a more stable complex with EDTA than does copper, and neither bismuth, EDTA, or the complex between the two absorbs light at the wavelength selected. After all the bismuth has been complexed, the EDTA will begin to form the strongly absorbing copper-EDTA complex, manifesting itself in the sloping portion of

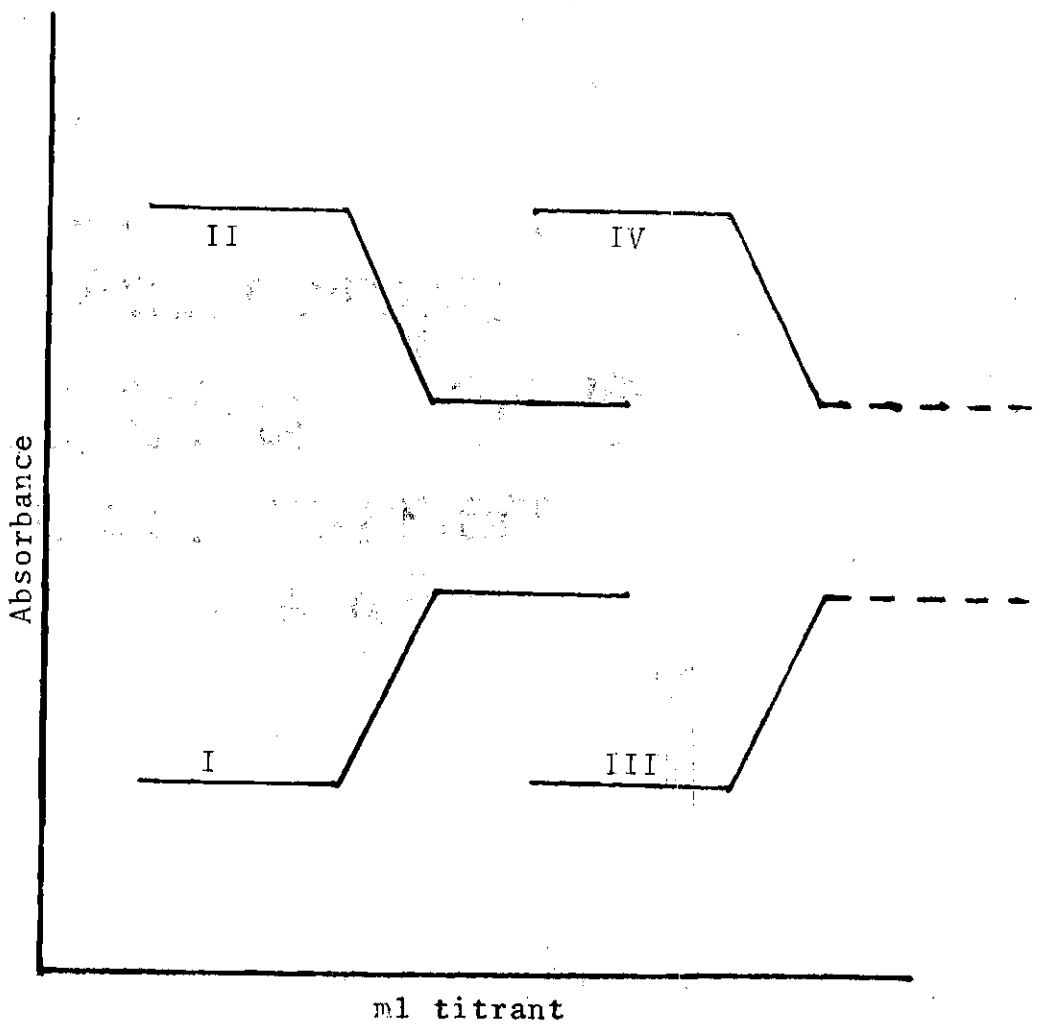

Figure 2. Shapes of Titration Curves when Using Step-Indication and Slope-Indication

the titration curve. The point to remember in this method of indication is that the indicator (copper) does not interact with the substance to be determined (bismuth). Suppose the titration is continued past the bismuth end point as indicated by the dotted line in Curve III of Figure 2. When all the copper has been titrated, the second horizontal line is formed in the titration curve, indicating the end point in the copper titration. Thus, if copper were already present in solution with the bismuth, the sequential titration of bismuth (using slope-indication) and copper (using self-indication) becomes possible.

A second example of slope-indication can be found in a paper by Flaschka and Sawyer [2]. If a small amount of Erio T is added to a solution containing calcium and magnesium, then only a step titration will result. The indicator will show the end point of both calcium and magnesium. If however, Erio T is added in excess of the magnesium present, then a self-indication system (magnesium-Erio T) is created that can be used for the slope indication of calcium with EDTA (Figure 2, Curve IV). Thus, if magnesium is already present in solution with the calcium, the sequential titration of calcium (using slope-indication) and magnesium (using self-indication) becomes possible.

Stability Constants

When titrating a metal "M^{2+}" with a chelon "Y^{4-}", the

reaction which takes place for most titration processes can be represented by the equation

$$M^{2+} + Y^{4-} \rightarrow MY^{2-} \qquad (3)$$

It is often useful for the analytical chemist to examine the equilibrium constant of equation 3 to determine if such a reaction meets the criteria of an analytical titration. The form of the equilibrium constant most useful to the analytical chemist, the stability constant, is expressed as

$$K = \frac{[MY^{2-}]}{[M^{2+}][Y^{4-}]} \qquad (4)$$

where the bracketed symbols represent the molar concentrations of the species in brackets.

The simplicity of equation 4 can be misleading unless one fully examines the system in which the reaction takes place. The value of $[M^{2+}]$ can differ drastically from the total analytical concentration of M due to the addition of species which also form complexes with the metal. The pH of the working solution can have a dramatic effect on the actual stability due to the protonation of the chelon.

The influence of such slide reactions can be taken into account by the use of the conditional stability constant which is expressed as

$$K_{cond} = \frac{[MY^{2-}]}{[M]^*[Y]^*} \quad (5)$$

where the asterisks indicate that the molar concentrations are the conditional ones.

The conditional stability constant will briefly be discussed here. Further reading on the conditional constant can be found in the informative monograph by Ringbom [4].

Influence of pH on $[Y^{4-}]$

Since chelons are weak polyprotic acids, they usually are only in the completely disassociated form in strongly alkaline solution. Generally, if the solution is below pH 10-11 then the concentration of Y^{4-} is much smaller than corresponds the total amount of Y added to the solution.

$[Y]^*$ can be determined as a function of $[Y^{4-}]$ from the relationship

$$[Y]^* = [Y^{4-}]\alpha_H \quad (6)$$

where

$$\alpha_H = 1 + K_1[H] + K_1K_2[H]^2 + \ldots + K_1K_2K_3 \ldots K_n[H]^n \quad (7)$$

K_1, K_2, K_3, ..., K_n are the step wise proton stability constants of the chelon and n is the degree of protonation of the chelon.

It is convenient for the chemist to make a plot of Log α_H vs. pH for quick reference. Figure 3 shows such a plot for EGTA. A computer program was written to calculate the α_H factor for any chelon and is discussed in Chapter V.

If protonation of the ligand is the only side reaction, the conditional stability constant can now be found from combination of Equations 4, 5, and 6, giving the expression

$$K_{\alpha H} = \frac{K}{\alpha_H} \tag{8}$$

Influence of Additonal Complex-Formers on $[M^{2+}]$

If a complex forming substance Z, (other than the titrant) is present then $[M^{2+}]$ will be considerably smaller than the total analytical concentration of metal added to the solution. The relationship between $[M^{2+}]$ and $[M]^*$ is expressed as

$$[M]^* = [M^{2+}]\beta_z \tag{9}$$

where

$$\beta_z = 1 + K_1[Z] + K_1K_2[Z]^2 + \ldots + K_1K_2K_3 \ldots K_n[Z]^n \tag{10}$$

K_1, K_2, K_3, ..., K_n are the step wise formation constants for MZ_n and n is the degree of complexation.

If the reaction between the metal M^{2+} and an

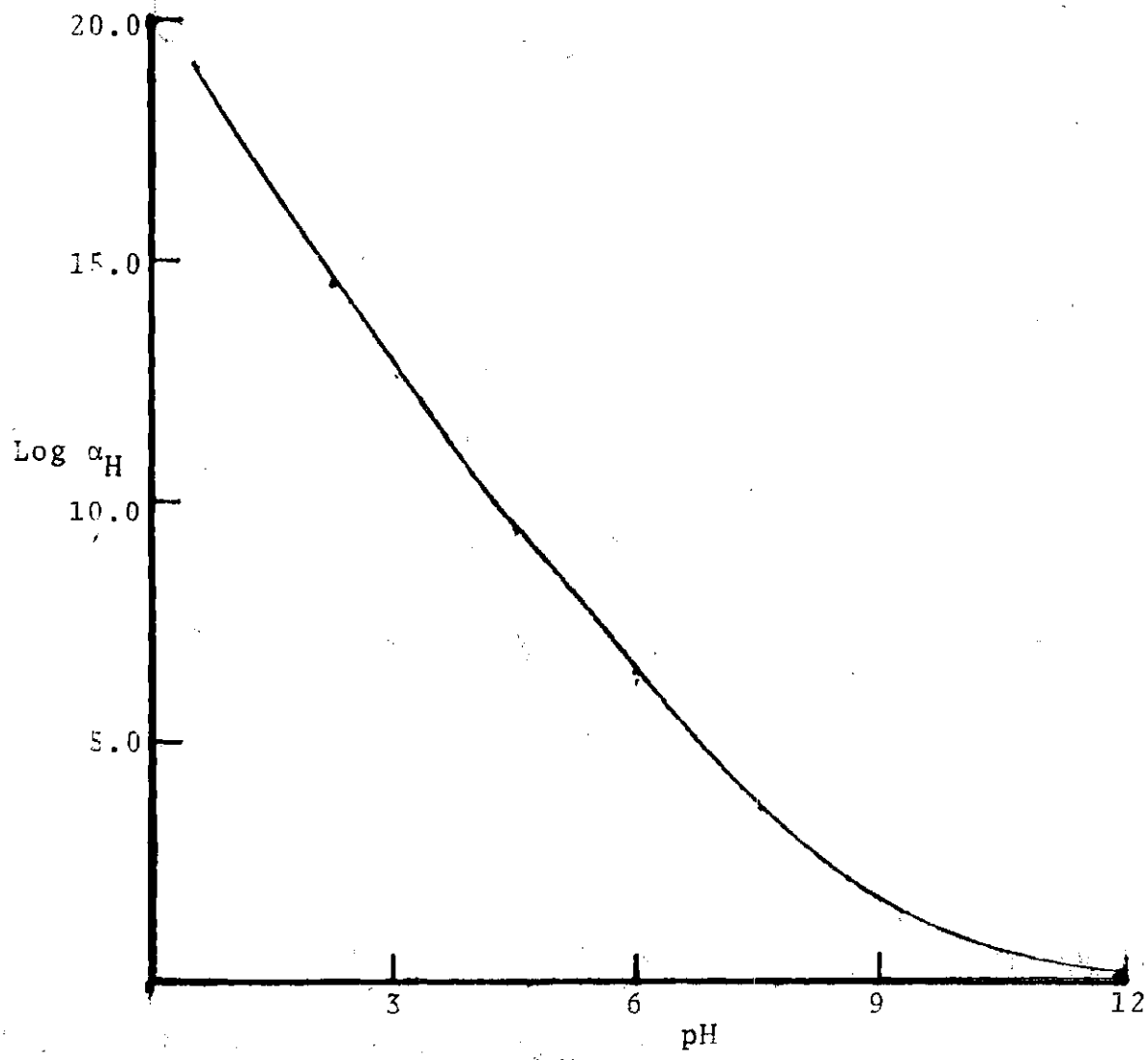

Figure 3. Log α_H vs. pH for EGTA

additional complex former Z is the only side reaction which need be considered, the conditional stability constant can now be found from combination of Equations 4, 5, and 9, and expressed as

$$K_{\beta_Z} = \frac{K}{\beta_Z} \tag{11}$$

If the additional complex former Z is the hydroxide ion, then the K's of Equation 10 are commonly referred to as the step wise hydrolysis constants of metal M. If the pH of the solution is allowed to become high enough to cause the formation of the metal hydroxide precipitate, the concepts must be expanded to include the solubility product constant of the metal hydroxide.

The effects of additional complex formers is a powerful tool in many analytical techniques. The majority of masking procedures are based on the presence of a substance that will lower the conditional stability constant of a reaction to such a level as to make it insignificant in the titration process.

It is important to remember that the calculation of β_Z is only valid for solutions in which Z is present in large excess to the amount of M in solution. As Equation 9 is a function of the uncomplexed Z, the portions of Z which is complexed with M may only be ignored if the amount of M is small in comparison to the total amount of Z present.

Combined Influence of Side Reactions on K

If more than one complex former is present in the solution, then the total β factor can be calculated as the sum of the individual β factors. Usually, only one β factor need be considered since it will be significantly larger than any of the other β factors which might enter into consideration.

If protonation of the ligand and the reaction between the metal M^{2+} and an additional complex former Z must both be considered, the conditional stability constant can now be found from combination of Equations 4, 5, 6, and 9, and expressed as

$$K_{\alpha,\beta} = \frac{K}{\alpha\beta} \qquad (12)$$

It must be remembered that the stability constants are only applicable if the particular reactions have reached equilibrium. If this is not the case, the conclusions drawn from the stability constant concepts are invalid.

CHAPTER II

DETERMINATIONS OF CALCIUM

Calcium is the fifth most abundant element in the earth's crust. It is found in numerous natural and artificial products, and thus its determination is a frequent task. In most products, calcium will be accompanied with magnesium in varying amounts. Because the chemistry of these two elements is similar, the determination of calcium in the presence of magnesium has always been a practically important and challenging problem.

Classical procedures for the analysis of calcium are tedious and time consuming. Before the introduction of EDTA, the titration with permanganate or the gravimetric determination were the most commonly used methods of analysis. Calcium oxalate is precipitated, separated by filtration, washed and dissolved in acid. The oxalate is then titrated with permanganate. Alternatively, the calcium oxalate is dried and weighed, or ignited and the calcium carbonate or calcium oxide is weighed.

One of the first procedures described for a complexometric titration was the determination of calcium with EDTA. Many indicators are available for this titration and function well as long as magnesium is not present in solution.

If magnesium is present, then serious problems arise in the determination of calcium.

The logarithms of the stability constants for calcium-EDTA and magnesium-EDTA are 10.7 and 8.7, respectively [5]. Unless masking of the magnesium is employed, the difference of 2 Log K units is too small to allow the titration of calcium by either instrumental or visual indication. Almost all common calcium indicators, that is, those that complex with the calcium, function properly only in highly alkaline solution. Fortunately, under these conditions, the magnesium precipitates as the hydroxide and thus masking is automatically provided. However, the titration is hampered by a decrease in the sharpness of the end point due to the presence of the precipitate. Furthermore, faulty results occur when the calcium is carried down by coprecipitation and escape titration.

As already stated, the above discussion is valid for the common type of indicators, namely, those that complex with the calcium. The magnesium-Erio T slope indicator for calcium mentioned in Chapter I, does not directly interact with calcium, and thereby allows the titration of calcium without the masking of magnesium. Its application, however, is severely restricted because the titration can only be used on the micro scale or lower, and does not allow large amounts of magnesium.

The introduction of EGTA (ethyleneglycol-bis(aminoethyl)-

tetra-acetate) as a titrant for calcium greatly improved the situation. The logarithms of the stability constants for calcium-EGTA and magnesium-EGTA as first reported by Schwarzenbach [6] are 11.0 and 5.7, respectively. The difference of 5.3 Log K units is large enough to allow the titration of calcium in the presence of magnesium without the necessity of masking the magnesium. However, the situation is only favorable when the indication process does not involve the complexation of calcium. Reiley et al. [7,8] reported a procedure for the potentiometric titration of calcium in the presence of magnesium with EGTA using the mercury drop electrode.

When trying to apply a calcium-complexing indicator, complications develop. First, any well working calcium indicator also reacts with the magnesium, and usually with a more pronounced color change. Thus, the selective titration of calcium in the presence of magnesium is impossible. Second, even if a good indicator is available, the situation is still very marginal. For the sake of explanation, let us assume that a good indicator is available which will only complex with the calcium and not magnesium. Then, when such an indicator is applied in the titration of calcium in the presence of magnesium with EGTA, the following situation prevails. The solution contains free calcium, free magnesium, and calcium bound to the indicator. Upon addition of titrant, the free calcium will first react. When all the free calcium

has been titrated, the next portion of titrant is supposed to take the calcium from the indicator complex, thereby causing a color change which indicates the end point. However, the calcium-indicator complex now imposes a β factor on the stability constant of calcium-EGTA. If the β factor is large, then the conditional stability constant of calcium-EGTA will be smaller than the magnesium-EGTA stability constant, and the end point will not occur until both the calcium and the magnesium have been titrated. If the factor is small, the calcium is taken from the indicator complex before the magnesium is titrated and the end point will occur at the right place. However, a small β factor means that the calcium-indicator complex is weak and this results in a bad end point. In principle, it is possible to remedy this situation by switching to a photometric end point. But even this should not work in the present case because the difference of 5.3 Log K units between calcium-EGTA and magnesium-EGTA is too small, and in addition, the absolute values of these stability constants are relatively low.

Procedures developed for this purpose have utilized slope-indication or indirect methods. A few of these will be discussed below. While they have not been as successful as desired, they will help to emphasize the problems associated with the titration of calcium and serve as a guide for further improvement.

Zn-EGTA-Zincon

Ringbom et al. [9] and Reiley et al. [10] described a procedure for the determination of calcium in solutions containing up to 30 times the amount of magnesium by weight. A solution stoichiometric in zinc and EGTA and a few drops of Zincon is added to the sample solution which is kept at pH 9.5 with ammonia-ammonium nitrate buffer. After mixing, the following reaction takes place

$$Ca^{2+} + \underset{\text{(yellow)}}{HIn^{3-}} + ZnEGTA^{2-} \rightarrow CaEGTA^{2-} + \underset{\text{(blue)}}{ZnIn^{2-}} + H^{+} \qquad (13)$$

Upon titration with EGTA, the free calcium is complexed first. After all the free calcium is titrated, the EGTA takes the zinc from the indicator complex and the color of the solution changes from blue to yellow. When the titration is followed photometrically, a curve such as that shown in Figure 2, Curve II, results. The end point of the titration is indicated by the second break in the titration curve since the amount of zinc-Zincon present in the solution is identical to the amount of calcium-EGTA which was formed before the titration was started.

While this method is highly successful under certain conditions, it is very sensitive to small changes in pH, buffer concentration, and amount of zinc-EGTA added. In order to prevent precipitation of zinc hydroxide, sufficient

quantities of complexing buffer must be added. However, if the buffer is allowed to reach concentration greater than 0.01 M, the sharpness of the color change is decreased seriously. Total zinc concentration must remain between 5×10^{-6} M and 2.5×10^{-4} M in order to obtain a color change adequate for an accurate determination.

Zn-EGTA-PAN

An indirect procedure for the titration of 1-4 mg calcium in the presence of up to 100 mg magnesium using zinc-PAN as the indicator has been described by Nakagawa, Wada, and Tanaka [11]. An excess of EGTA is added to the buffered (pH 8) sample solution. After addition of a solution stoichiometric in zinc and EGTA, and the addition of a PAN solution, the excess EGTA is titrated with standard calcium. The end point is indicated by the appearance of the red color due to the zinc-PAN complex.

Pan and its complexes are water insoluble and present in the solution in colloidal suspension. Because of this, a serious disadvantage of this method is the necessity of working very slowly, and of warming and vigorous stirring when approaching the end point.

Cu-NH_3-EGTA

A procedure has been described by Reiley et al. [12] for the titration of calcium in ammoniacal solution with EGTA using copper as the slope indicator. Since copper forms a more stable complex with EGTA than calcium, it is

necessary to impose a β factor on the stability constant of copper-EGTA. By plotting the logarithm of the conditional stability constant of the EGTA chelonates of copper, calcium, and magnesium vs. the negative logarithm of the ammonia concentration (pNH_3), the proper concentration of ammonia was estimated. It was found that an ammonia concentration of 0.3 M (pNH_3 0.5) reduces the conditional stability constant of copper-EGTA to approximately 3.6 Log K units below that of calcium.

Thus, the EGTA initially added to the solution complexes with calcium. After all the calcium has been titrated, EGTA takes copper from the ammonia complex, thereby decreasing the abosrbance and providing the break in the titration curve necessary for the location of the calcium end point (Figure 2, Curve III).

A serious disadvantage of this method is the lack of sensitivity which results from the relatively low absorptivity of the copper-ammine complex. An uncertainty of ± 4.0% was reported at the 1 mg calcium level.

Discussion

The type of indicator systems which have been developed for the titration of calcium with EGTA, representative samples of which have been given, have been successful but with room for improvement. For instance, if a complex with high absorptivity could be substituted for copper-ammonia in the above titration, an increase in sensitivity would be

obtained. Since PAR [4-(2-pyridylazo)resorcinol] forms highly absorbing, water soluble complexes with many metals, it was felt that investigations should be made into the use of some of these complexes as slope indicators for the titration of calcium with EGTA.

Since most metal-PAR complexes have a rather high stability, a large β factor will be imposed on the metal-EGTA stability constant. Since the size of this factor can be influenced by the concentration of PAR, solvents, pH, and buffer, the possibility exists of using these parameters to influence the metal-EGTA stability constant in such a way as to obtain an indicator system of low sensitivity with respect to magnesium. However, with the stability constants of calcium-EGTA and magnesium-EGTA being relatively low, and the difference between these constants being small, the probability of success was not very high, but certainly high enough to warrant theoretical and experimental investigations.

CHAPTER III

CALCULATIONS

This work was started in order to find a metal-PAR complex which would act as a slope indicator for the titration of calcium with EGTA. Ultimately, it was hoped that such an indicator would also allow the titration of calcium in the presence of magnesium.

Calculations were performed to obtain the conditional stability constants for the EGTA complexes of several metals at different concentrations of PAR and auxiliary complex formers. The calculations and their implications are discussed below.

Calculations of the Conditional Stability Constants

The stability constants and physcochemical data for the 1:2 PAR complexes of divalent metals have been well studied and reported. Relevant data for some metal-PAR complexes have been taken from summarizing works and compiled in Table 1 [13].

By applying the formulas derived in Chapter I to the data shown in Table 1, the conditional stability constants for the metal-EGTA complexes at different concentrations of PAR were obtained. A computer program, detailed in Chapter V was written to calculate these quantities. The results of

Table 1. Physcochemical Data and Logarithms of Stability Constants for Selected PAR Complexes [13]

Metal	Log K_1	Log K_2	λ_{max}, mμ	Color	Absorptivity at λ_{max} lit/mole-cm
Cu^{2+}	14.8	9.1	510	Red	5.89×10^4
Pb^{2+}	12.9	13.7	522	Red	5.02×10^4
Mn^{2+}	9.7	9.2	500	Red	Not Available
Cd^{2+}	11.5	10.1	510	Red	1.02×10^4
Zn^{2+}	12.6	12.7	495	Pink	6.34×10^4
Co^{2+}	10.0	7.1	510	Red	5.6×10^4

these calculations are summarized in Table 2.

When considering the data in Table 2, and their applications, it should be remembered that the conditions assumed when calculating these values might be different from the conditions existing when actually performing a titration. While the calculated value is a good guide in research, and much information can be derived from it, small differences in the conditions when titrating, which might be insignificant individually, can combine to give a sizable deviation form expected behavior.

For instance, when using Equation 10 to calculate β_Z, where Z is PAR, the conditional stability constant of the metal-PAR should be used. However, for the particular case at hand, namely the titration of calcium, the α_H for PAR, which is a weak, diprotic acid, has not been considered. The reason for this is that in aqueous medium, calcium determinations must be done in alkaline solution, and for pH greater than 6, the α_H for PAR is very close to one. It should also be remembered that Equation 10 is only valid for solutions in which Z (PAR) is in large excess over the metal under consideration. Under this condition, the amount of PAR which is bound to the metal is not significant in comparison to the total amount of PAR is solution. This condition is difficult to meet in practice since PAR is not soluble enough, and even if it were, the resulting solution would be so optically dense as to make photometry impossible.

Table 2. Logarithms of Conditional Stability Constants of Metal-EGTA Complexes at Different Conc. of Z Log K, Log K_{EGTA} Data from Reference 5

Metal	LogK_{EGTA}	Z	Log K_{cond} at PZ									
			1	2	3	4	5	6	7	8	9	10
Pb^{2+}	14.8	PAR	-9.9	-7.9	-5.9	-3.9	-1.9	.08	2.1	4.1	6.1	8.1
Mn^{2+}	12.3	PAR	-4.7	-2.7	-.7	1.3	3.3	5.3	7.3	9.3	11.0	12.0
Cd^{2+}	16.7	PAR	-3.0	-1.0	.98	3.0	5.0	7.0	9.0	11.0	12.9	14.7
Zn^{2+}	12.9	PAR	-2.3	-.32	1.7	3.7	5.7	7.6	9.1	10.3	11.3	12.2
Co^{2+}	12.3	PAR	-2.9	-.92	1.1	3.1	5.1	7.0	8.8	10.1	11.1	11.9
Cu^{2+}	17.8	PAR	-4.2	-2.2	-.22	1.8	3.8	5.8	7.8	9.7	11.5	12.8
Cu^{2+}	17.8	NH_3	9.3	13.1	15.9	17.3	17.6	17.7	17.7	17.7	17.7	17.7

A further influence will be exerted if the solution contains substances which form very weak complexes. Although these effects will be small, they will still contribute to the final behavior of the system.

In addition, values of stability constants are dependent on ionic strength and temperature, and these parameters may differ for the situation under which the stability constants were determined and those under which titrations are actually performed. Furthermore, literature values may also differ according to the method employed when determining the constants. When available, only values obtained from spectrophotometric data were employed.

Finally, it should be mentioned that judgments based on the data from Table 2 should only be applied to solutions in which full equilibrium has been reached. No matter how accurate the stability constant, it can never predict kinetic behavior. In practice, one sometimes operates with solutions in which full equilibrium has not been attained. This is perfectly legitimate however, since these effects will often be small. Thus, while this and the previously mentioned factors may have a small influence on the behavior of a titration reaction, in many cases too small for individual consideration, the sum of these factors can become significant. The difficulty is that the data required to determine these influences are not available, and even if they were, the amount of mathematical work involved would be

tremendous. Consequently, it is best to act according to the old saying, "Theory guides, experiment decides."

Conclusions Based on the Calculated Data

As expected, the influence of the strongly complexing PAR on the stability of the EGTA complexes of the metals intended as slope indicators is dramatic. An ideal situation would be to have a value of the conditional stability constant for the metal-EGTA which is between those of calcium-EGTA and magnesium-EGTA. As shown in Table 2, this condition is only met when the PAR concentration is between 10^{-8} and 10^{-6}F. Since the PAR concentration must be greater than that of the indicating metal, the concentration of the actual indicating system (metal-PAR) would be considerably lower than that calculated for PAR.

The low concentration of indicating complex results in two immediate problems. The first is related to absorbance. While most metal-PAR complexes have an absorptivity in excess of 10^4 1/cm-mole, at the concentrations mentioned above, long-path phototitrators would be required in order to obtain readings of sufficient magnitude. The second is related to the establishment of the titration curve. In attempting a photometric titration using a slope-indicator, there must be sufficient indicator present to allow establishment of the sloping line. For this purpose a minimum of three points must be obtained, which means three additions

of titrant. At the concentrations of indicating complex mentioned above, even with a very dilute titrant solution, one single drop of titrant will completely consume the indicating metal.

CHAPTER IV

EXPERIMENTS

With the data of the conditional stability constants from Chapter III as a basis, it was realized that a metal-PAR complex could be used as a slope-indicator for the titration of calcium alone. In the very moment that magnesium is present in the solution, a very marginal situation exists that can only be resolved by delicate juggling of pH, solvent, and buffer. Thus, experiments were performed to ascertain which metal-PAR complexes work correctly for the determination of calcium in magnesium free solutions. Afterwards, the experiments were expanded to find for which of these systems the conditions could be modified to decrease this sensitivity towards magnesium.

All equipment and chemicals used in these experiments are described in the following section. A detailed procedure for the titration of calcium using copper-PAR as the slope indicator is given below. The following general comments apply to all the described experiments.

During the course of the investigation, it was often necessary to change the pH and other parameters of the solution in the midst of a titration. Because of this, the titrations were performed in a 50-ml beaker: this permitted

the use of a large enough volume of solution to allow visual observations, and insertion of the pH meter electrodes. For the optical measurements, a portion of the solution was transferred to the cuvette. Special care was taken that this portion was representative of the solution in the titration vessel.

While many of the metal-PAR complexes did not perform well as a slope-indicator, many observations, interesting in this context, were made and therefore will be discussed briefly.

Chemicals and Equipment

Chemicals

Distilled water was obtained from a Barnstead still equipped with a Ventguard filter. The water was then passed through a mixed bed deionizer. Water prepared in this way was used exclusively for preparing and diluting reagents and solutions. All common acid, base, and buffer solutions were prepared from reagent grade chemicals. Metal salt solutions were prepared from J. T. Baker "Analyzed Reagent" grade salts, except for indium chloride which was prepared by dissolving the metal in concentrated hydrochloric acid. J. T. Baker "Analyzed Reagent" grade PAR was used. Eastman analytical grade EGTA was used exclusively for preparation of the standarized titrant solutions.

Glassware. Class A glassware was used throughout

without additional calibrations. Common laboratory glassware such as beakers and flasks were used as needed.

pH Meter. All required pH measurements were made with a Corning Model 7 pH meter. This instrument was calibrated with 0.01 molal Borax solution (pH 9.2).

Spectrophotometers. All spectrophotometric measurements used for the titration curves were obtained with a Bausch and Lomb Spectronic 20. If absorbance curves were required, a Bausch and Lomb Spectronic 505 Spectromphotmeter was used.

Results of Experiments

Many metal-PAR complexes were investigated as possible slope-indicators. As feared, the neglection of some parameters in the calculations caused discrepancies between predicted and actual behavior.

For instance, the data from Table 2 leads one to believe that lead-PAR would perform well as an indicator for a calcium titration. The experimental evidence, however, did not verify this fact. Good titration curves are not obtained because the indicator metal and calcium react simultaneously.

This problem becomes readily apparent when examining Figure 4. Curve I of Figure 4 is a titration curve for lead-PAR alone. Curve II is for the same indicator in a solution with 10 μg calcium. As can be seen in Curve II, there is no break in the curve, and the slope of the line is less negative than that for the titration in the absence of

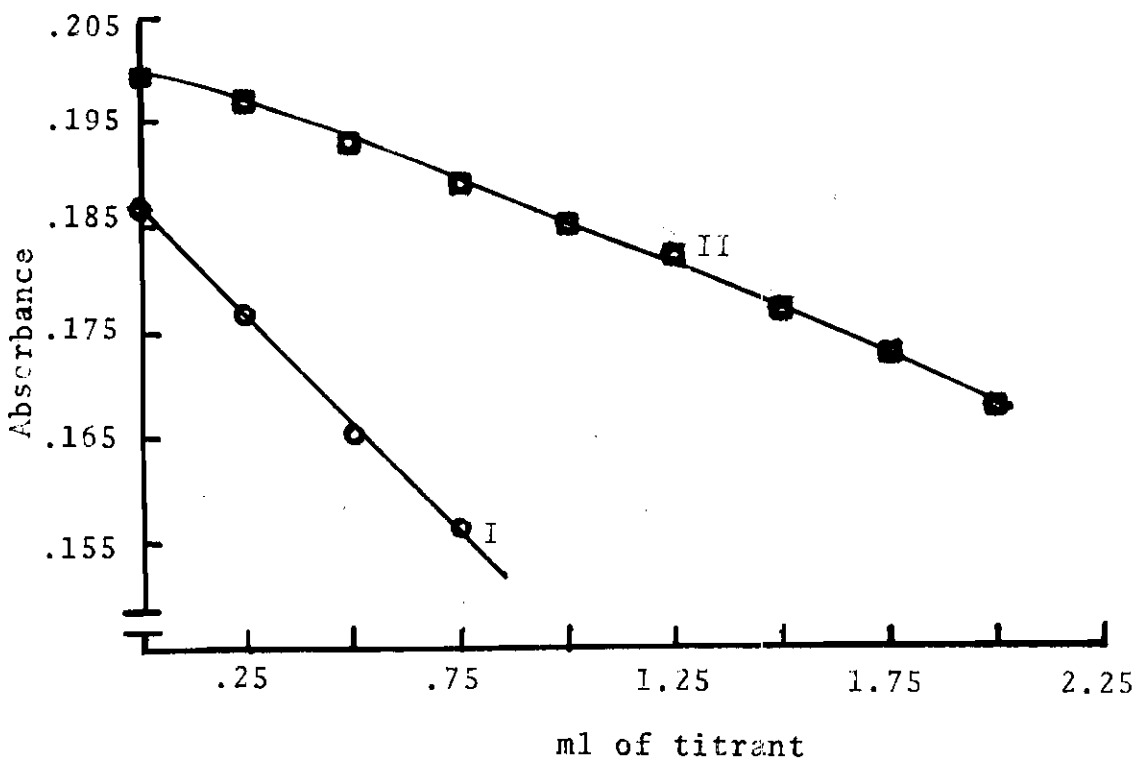

Figure 4. Titration of Lead-PAR. (Curve I is for a calcium free solution, Curve II for a solution containing 10 µg calcium.)

calcium. This is very unfortunate because with regard to magnesium, the indicator is quite suitable. When the titration leading to Curve I was repeated, but in the presence of 20 µg magnesium, there was essentially no change in the curve.

Because of this, several attempts were made to improve the situation with regard to calcium by either decreasing the stability of the lead-EGTA or increasing the stability of the calcium-EGTA. First, the concentration of free PAR was increased with the expectation that the lead would then show less tendency to complex with the EGTA. This approach was futile and experiments were ended when the free PAR concentration reached levels in which the solution became so optically dense that photometry was impossible. Second, the addition of alcohol to the titrated solution was attempted. It is known that the stability of most chelonates is increased in media consisting of water and organic solvents. It was hoped that this increase would be more pronounced for calcium-EGTA than lead-EGTA. Unfortunately, the addition of ethanol or methanol to an extent to make the working solution 50% v/v in alcohol did not result in an improvement in the titration of calcium.

The data from Table 2 also lead one to believe that the PAR complexes of cadmium, manganese, zinc, and cobalt, all in the divalent state, would act as good indicators for the titration of calcium. However, in the cases of cadmium,

manganese, and zinc, the PAR complexes react simultaneously with calcium, thereby giving curves similar to Curve II of Figure 4. Cobalt-PAR is not attacked by EGTA. This is probably due to the fact that even though cobalt (II) was originally prepared, by the time experiments were performed the cobalt had oxidized to cobalt (III), the PAR complex of which is too stable to react with EGTA.

Indium-PAR was investigated next as a possible indicator, but on an experimental basis only. Preliminary calculations could not be run for this system because a stability constant for indium-EGTA could not be found in the literature. Even so, it was still felt that indium was a good alternate trivalent metal for the investigation.

In attempting to use indium, the hydrolysis of the indium and its precipitation as the hydroxide proved to be a severe problem. It was found that the maximum pH allowable in which the indium-PAR would not decompose was about 8.5. Under these conditions, problems similar to those experienced with lead-PAR were experienced. Calcium and the indicator react at the same time with the titrant and no improvement was found by changing buffer, pH, or solvent.

Of the many elements investigated, only copper was found to form a complex with PAR which will act as a slope-indicator. Because of this, further experimentation with other metals was stopped and full effort was given to find the optimal conditions for using copper-PAR. Good results

are obtained with this indicator for solutions containing from 4 to 200 µg calcium. The titration is easy to perform and gives excellent curves. However, it was found that if more than 2 µg of magnesium is present, then some portion of the magnesium reacts to the titrant simultaneously with the indicator, and error can occur in the determination.

Attempts were made to decrease the sensitivity of the titration to magnesium by careful adjusting of pH, buffer, and solvent. For example, it was felt that changes in the buffering system might help this problem since ammonia does form a stable complex with copper, and therefore changes the conditional stability of copper-EGTA. The concentration of ammonia was lowered while keeping the pH at 10. The ammonia concentration was also varied after lowering the pH of the solution by the addition of sodium acetate. The complexing ammonia buffer was also completely eliminated and a borax buffer was used to maintain pH. None of these changes improved the situation with regards to the amount of magnesium tolerable. Therefore, in a final effort, the procedure originally used for this titration was refined to work with calcium alone. This procedure is given below.

Procedure for the Titration of Calcium with EGTA Using Copper-PAR as the Slope-Indicator

(1) Adjust the spectrometer for 0% transmittance with the shuuter in front of the photodetector. Then adjust the instrument for 100% transmittance with the cuvette, which

will be used for measurements, filled with water and placed in the cell holder.

(2) Pipet a sample containing from 4 to 200 μg calcium into a beaker of adequate size.

(3) Add 5 ml of 10^{-4} F PAR and 1 ml of 10^{-4} F copper to the solution.

(4) Add sufficient ammonia-ammonia chloride (pH 10) buffer to make the solution 0.1 F in ammonia.

(5) Titrate with 10^{-4} F EGTA. After each addition of titrant, stir vigorously and then place a portion of the solution into a Spectronic 20 cuvette. (See note 1).

(6) Record the applicable measurement, being sure to correct readings for dilution.

(7) Before adding another portion of titrant, return the solution in the cuvette to the main portion in the beaker, being careful to avoid loss.

Note 1: In order to avoid small amounts of the solution from dripping down the side of the glassware when making transfers, it is advisable to place a small amount of stopcock grease along the open end of the cuvette and at the pour spout of the beaker.

Evaluation of the New Method

When following the above procedure, good titration curves such as that shown in Figure 5, for solutions containing no magnesium, are obtained. Numerous titrations were performed and a representative sample of the results is

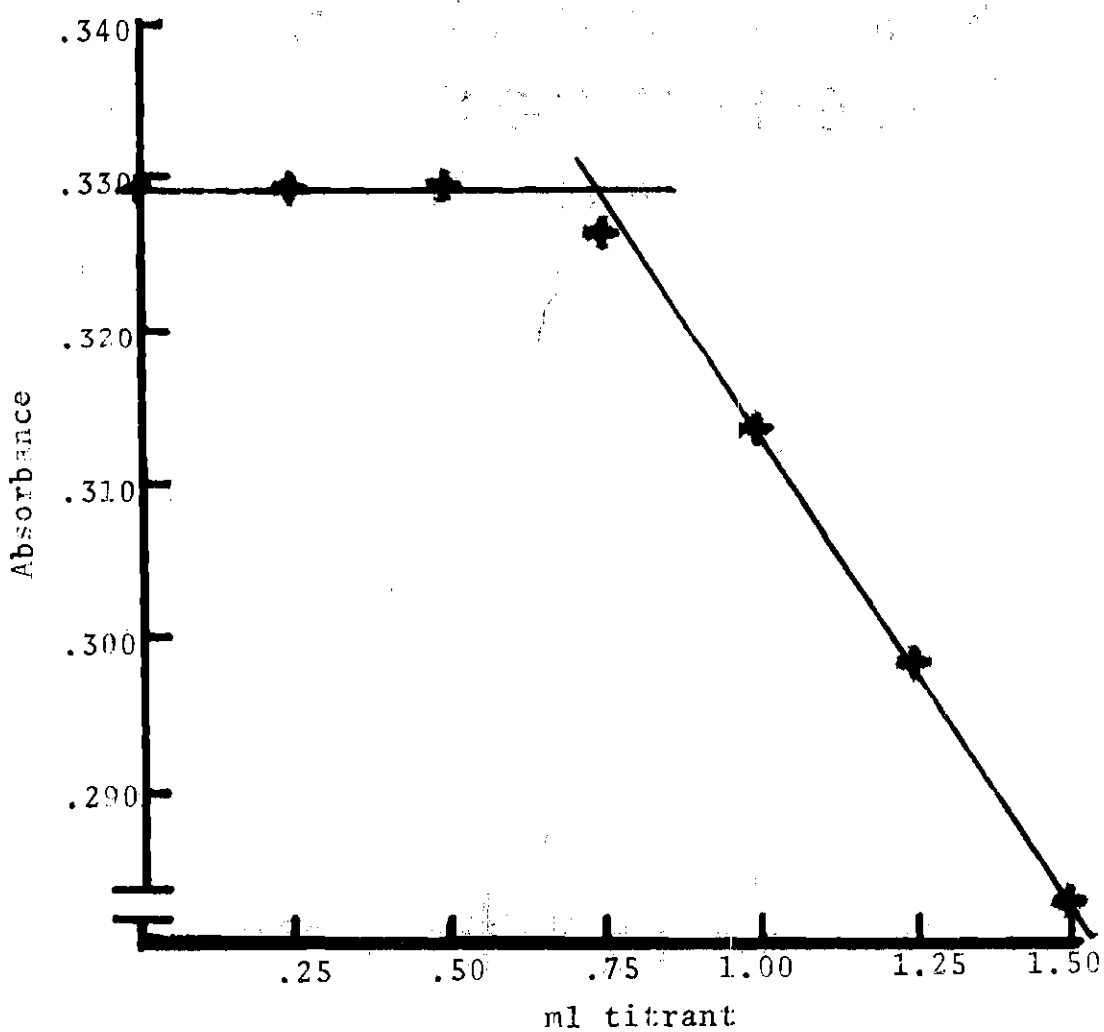

Figure 5. Titration of 4 µg Calcium
(No magnesium present)

given in the first half of Table 3. As can be seen, the results are both accurate and precise.

Interference from heavy metals must be considered when applying this method in practice. Techniques for eliminating these interferences, such as sulfide precipitation or the introduction of masking agents are well established and should be readily applicable to the new method.

However, of greatest interest is the interference due to magnesium. The second half of Table 3 contains results obtained for various amounts of calcium in the presence of magnesium. As can be seen from this data, good results are obtained for solutions containing up to 2 μg of magnesium. When more than 2 μg of magnesium are present in solution, then error will occur. A study of the relevant titration curves is of interest. Figures 6 and 7 show the titration curves for 4 μg calcium in the presence of 2 and 4 μg magnesium, respectively. As can be seen from these curves, the end points of the titrations are identical for solutions containing 0 and 2 μg magnesium, but a 15% error occurs for the solution containing 4 μg magnesium. Even more curious are the results obtained for solution containing larger quantities of magnesium where a negative error then occurs. Figure 8 shows the curve for the titration of 100 μg calcium in the presence of 25 μg magnesium. As can be seen, a considerable rounding of the curve takes place in the area

Table 3. Representative Results for the Titration of Calcium with EGTA Using Copper-PAR as the Slope-Indicator

µg Calcium Taken	µg Calcium Found	Δ	µg Magnesium Present
4.0	4.1	+0.1	None
4.0	4.0	0.0	None
40.0	38.6	-1.4	None
100	101	+1.0	None
200	196	-4.0	None
4.0	4.1	+0.1	1.0
4.0	4.1	+0.1	2.0
4.0	4.6	+0.6	4.0
100	82	-18	25
200	174	-26	25

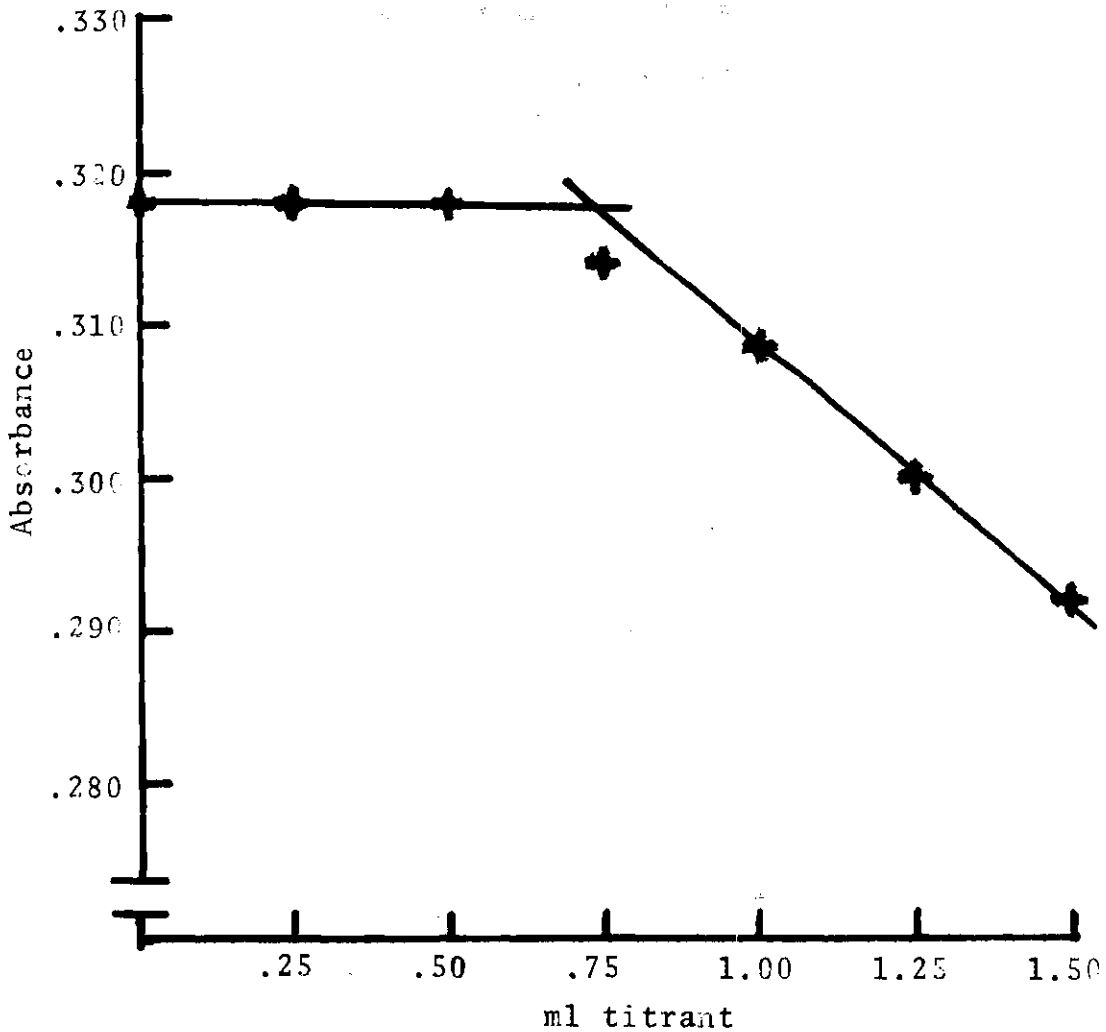

Figure 6. Titration of 4 µg Calcium in the Presence of 2 µg Magnesium

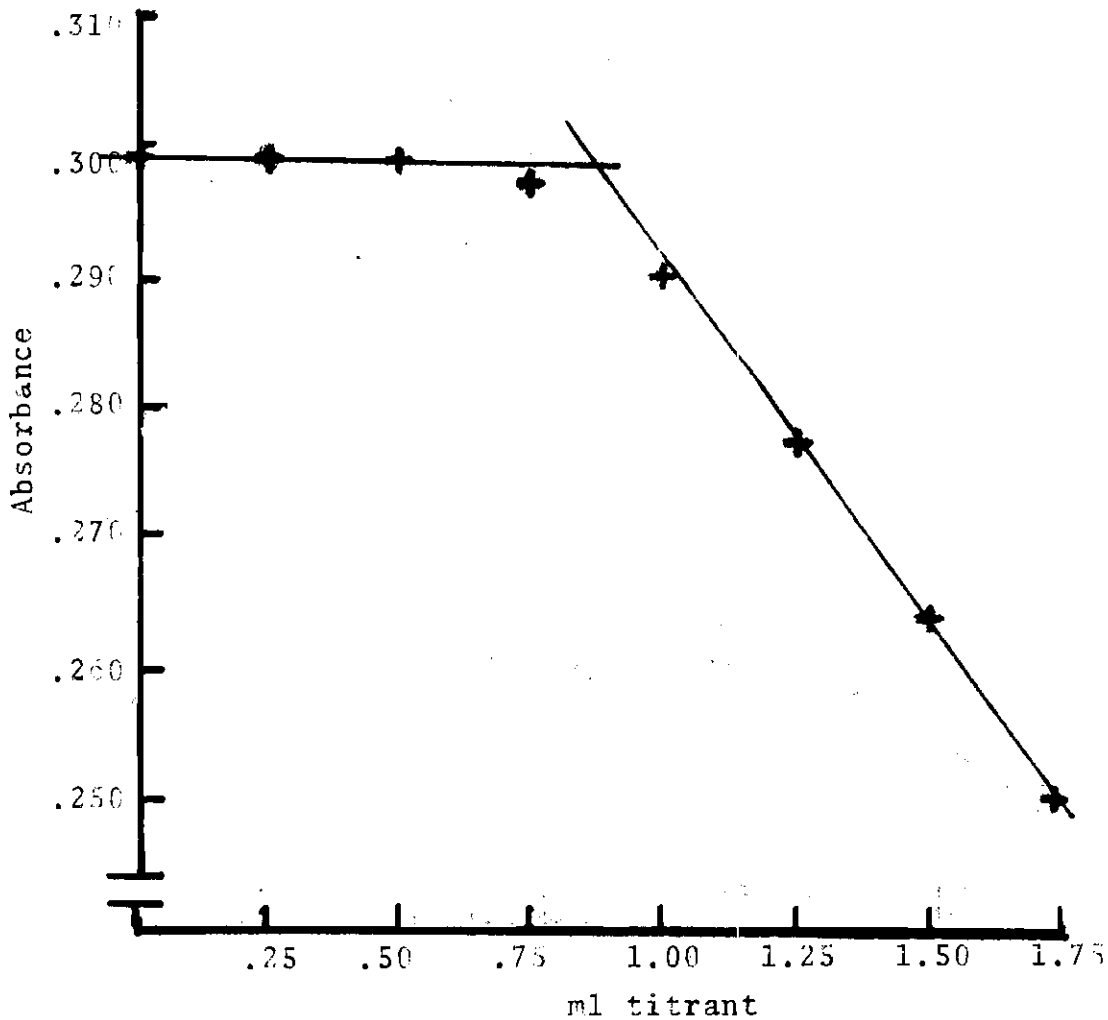

Figure 7. Titration of 4 µg Calcium in the Presence of 4 µg Magnesium

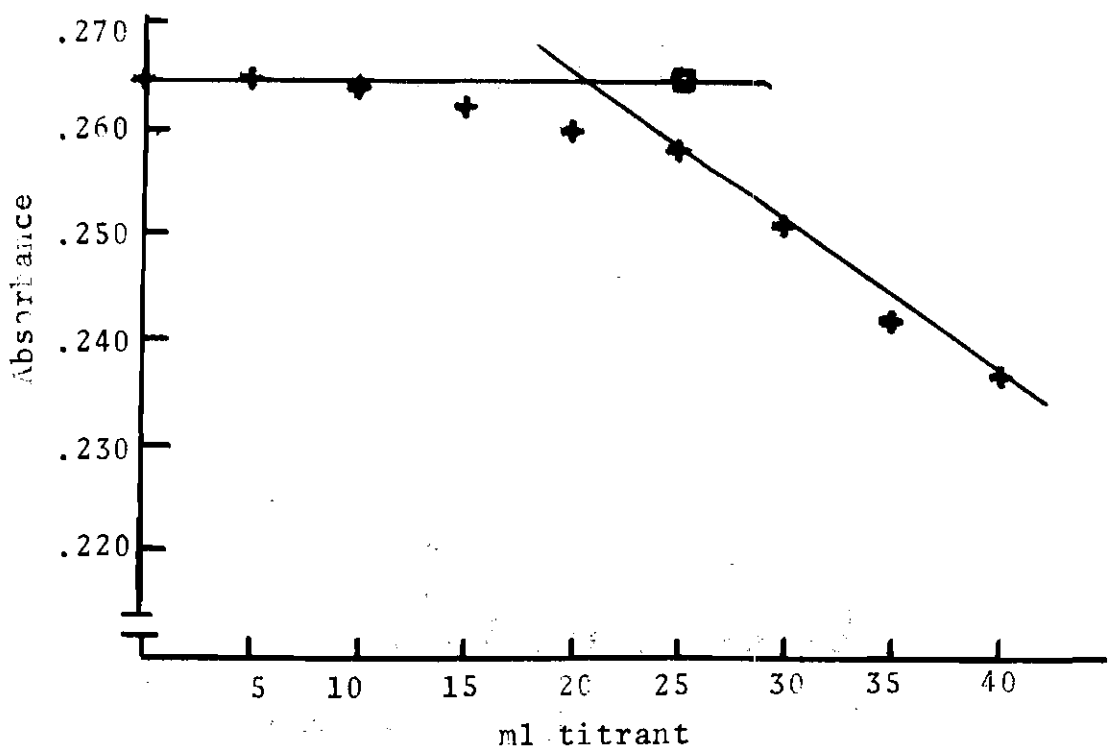

Figure 8. Titration Curve for 100 µg Calcium in the Presence of 25 µg Magnesium. (Square indicates expected location of end-point.)

of the end point and the portion of the titration curve corresponding to the reaction between the indicator and titrant is less negatively sloping than the corresponding curves for solutions containing no magnesium. Therefore, when extrapolating the straight line portions of the curve, intersection occurs far to the left of the correct end point.

It is impossible to give a statement concerning the maximum amount of magnesium which is allowable. This amount depends on the conditions under which the titration is performed, and of course, greatly on the error that is acceptable.

CHAPTER V

COMPUTER PROGRAMS FOR CALCULATING CONDITIONAL STABILITY CONSTANTS AND α FACTORS

Relatively inexpensive but very powerful computers have become available due to recent developments in the field of electronics. Because of their applications, small physical size, small instruction set, and small amount of core memory, these computers have been commonly referred to as "mini-computers." They are now common place in academic and industrial laboratories.

There are two common functions for the mini-computer. The first is in the area of data acquisition. Because of their low price, and advances which have been made in the areas of data transmission and analog to digital conversion, mini-computers are a cost effective and statistically accurate means of obtaining real-time data from a wide variety of instruments. Secondly, the mini-computer has become an efficient means of processing data and performing tedious and complicated calculations in a very short time. Advances in soft-ware have allowed powerful and easily comprehended programming languages to be run on these devices.

The calculations performed for Chapters I and III of

this report were done with such a computer. A description of the programs written and how they may be modified to include additional parameters is given below.

Computer and Programming Language

The computer used for these programs is a Digital Equipment Corporation PDP 1ab8/e with 8k words of core memory. The programming language is Basic RT where RT indicates "real time." The computer is equipped with a CRT and paper tape reader and punch. Both of these devices can be utilized with the proper commands in Basic.

Programs

Program 1, which is listed below, is used to calculate the α_H factor for any chelon according the Equation 7. The calculation is done for a pH range of 1-14. Figures 9 and 10 are the flow charts for the important segments of Program 1 and should be examined in sequence. The following comments will be helpful in understanding this program.

Lines 1-3 are remark (REM) commands and have no effect on the sequence of events which takes place. Because stability constant data is usually available as the logarithm of their value, the program will request the logarithm of the proton stability constants and then take the antilog of this value at line 16. If the stability constant that is given to the computer is not a logarithm, then line 16 must be eliminated from the program. Once all the information requested is

```
1 REM PROGRAM FOR DETERMINING THE "ALPHA" FACTOR OF ANY MULTIPROTONATED
2 REM CHELON. REQUEST FOR K(A) SHOULD BE GIVEN AS THE LOG OF THE
3 REM FORMATION CONSTANTS
10 PRINT "DEGREE OF PROTONATION"\INPUT H
15 FOR A=1 TO H\PRINT "LOG K("A")="\INPUT K(A)
16 K(A)=10↑K(A)
17 B(A)=1
18 FOR R=1 TO A\B(A)=B(A)*K(R)
19 NEXT R
20 NEXT A
22 PRINT \PRINT \PRINT
23 PRINT "PH","ALPHA","LOG ALPHA"
24 PRINT \PRINT
25 FOR P=1 TO 14 STEP .1
26 A=1
30 FOR R=1 TO H
35 A=A+((10↑(-P)↑R))*B(R)
40 NEXT R
45 PRINT P,A,LOG(A)/LOG(10)
65 NEXT P
```

Program 1. Calculates α_H for any Chelon in a pH Range of 1-14

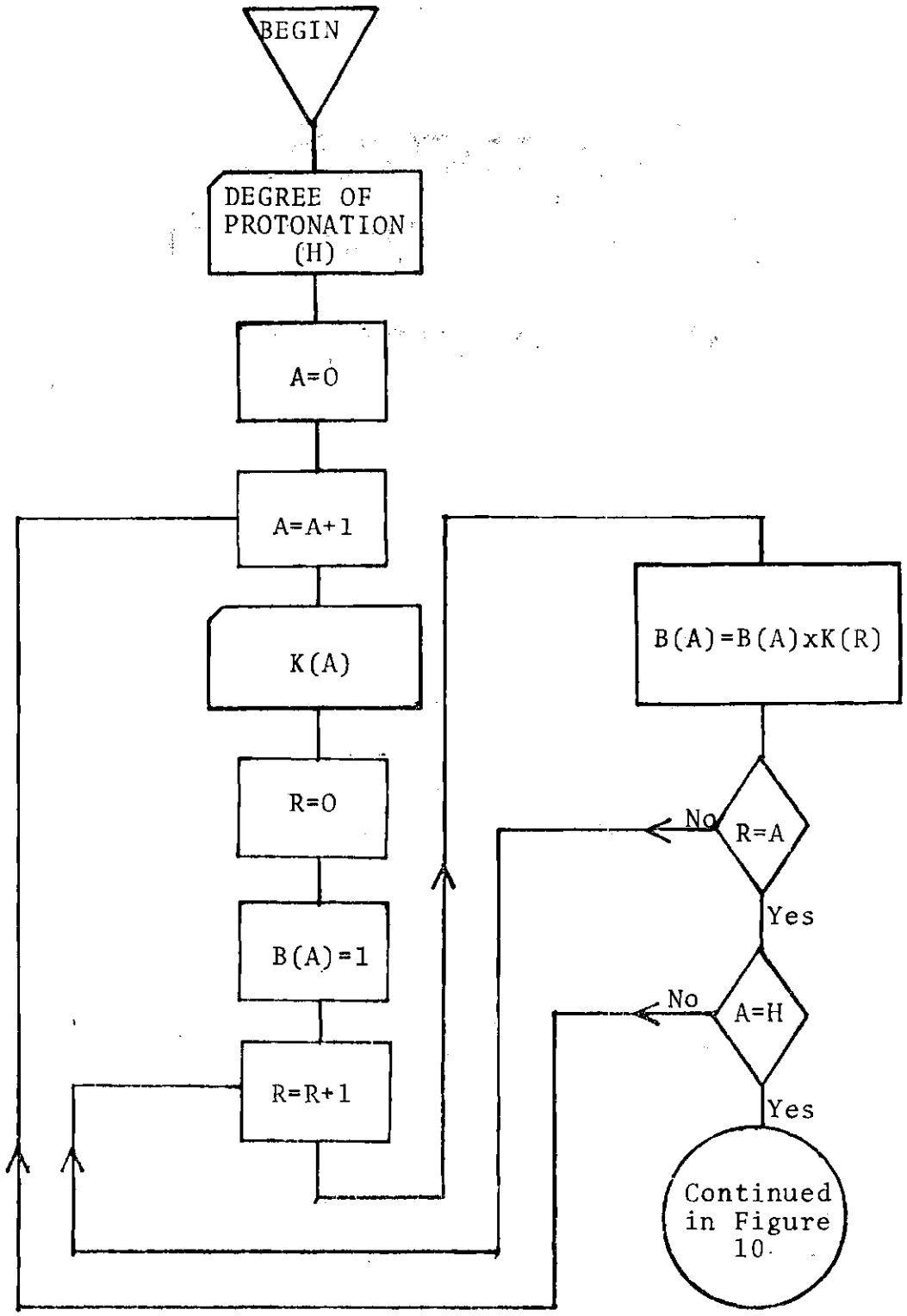

Figure 9. Flow Chart for Program 1

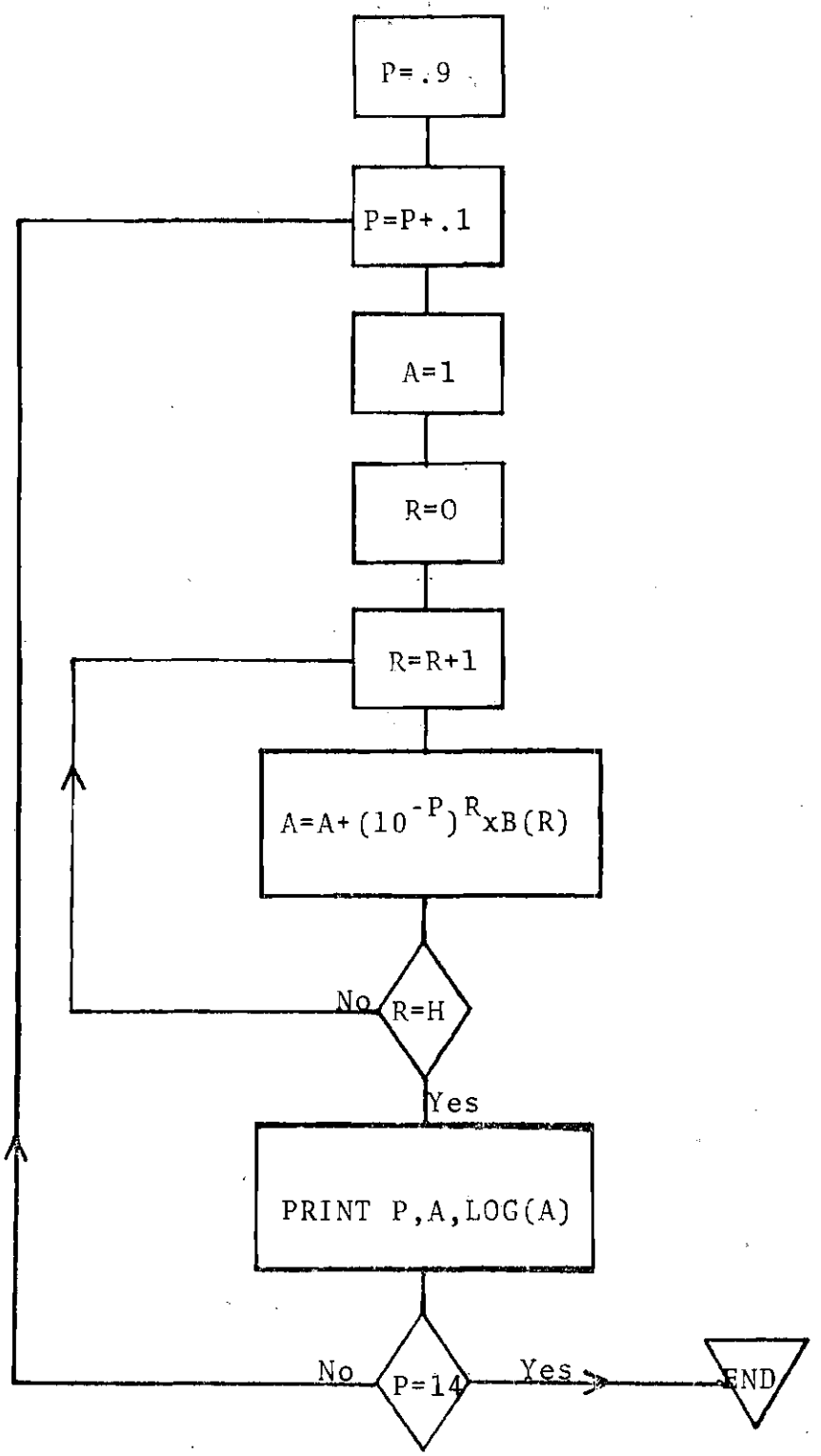

Figure 10. Continuation of Flow Chart for Program 1

supplied to the computer, the teletype will print the proper headings and then give the value of pH, and the calculated value of α_H and Log α_H. The .1 pH unit increment is probably too narrow for any practical use and can easily be changed at line 25. The use of the necessary plot commands within lines 25-65 will cause the computer to display the data in graphic form on the CRT.

Program 2, which is listed below, is used to calculate the conditional stability constant of a metal chelonate in the presence of an additional complex former according to the equation

$$\text{Log } K_{\text{cond.}} = \text{Log } K - \text{Log } \alpha_H - \text{Log } \beta_Z$$

The calculations are done for a pZ range of 1-10. Figures 11, 12, and 13 are the flow charts for the important segments of Program 2 and should be examined in sequence.

Since the format of the calculations of α_H and β_Z are identical, the same subroutines at lines 100-117 and 120-135 can be used in these calculations. The α_H factor for the additional complex former may also be included by using these subroutines, storing the calculated value, and using this value at the proper location within line 65. If the .1 pZ increment is unnecessarily small for the purposes of the user, it may be changed at line 55.

```
1 REM PROGRAM FOR DETERMINING THE CONDITIONAL STABILITY CONSTANT
2 REM OF ANY METAL CHELONATE.  REQUEST FOR K'S SHOULD BE GIVEN
3 REM AS THE LOG OF THE FORMATION CONSTANT OR THE LOG OF THE ACID
4 REM STABILITY CONSTANT RESPECTIVELY. THIS PROGRAM DOES NOT
5 REM INCLUDE THE BETA FACTOR DUE TO THE HYDROLYSIS OF THE
6 REM METAL OR THE ALPHA FACTOR DUE TO THE PROTONATION OF THE
7 REM ADDITIONAL COMPLEX FORMER.
20 PRINT "PH="\INPUT P
25 PRINT "LOG STABILITY CONSTANT FOR  MY="\INPUT S
30 PRINT "DEGREE OF PROTONATION OF Y="\INPUT H
35 GOSUB 100
37 GOSUB 120
39 PRINT \PRINT
40 X=A\PRINT "ALPHA="X,"LOG ALPHA="LOG(X)/LOG(10)
41 PRINT \PRINT
45 PRINT "DEGREE OF COMPLEXATION OF MZ"\INPUT H
50 GOSUB 100
52 PRINT \PRINT \PRINT "PZ","LOG K","LOG BETA"\PRINT
55 FOR P=1 TO 10 STEP .1
60 GOSUB 120
65 PRINT P,S-(LOG(X)/LOG(10))-(LOG(A)/LOG(10)),LOG(A)/LOG(10)
70 NEXT P
80 STOP
100 FOR A=1 TO H\PRINT "LOG K("A")="\INPUT K(A)
105 K(A)=10↑K(A)\B(A)=1
110 FOR R=1 TO A\B(A)=B(A)*K(R)
115 NEXT R\NEXT A
117 RETURN
120 A=1\FOR R=1 TO H
125 A=A+((10↑(-P)↑R))*B(R)
130 NEXT R
135 RETURN
```

Program 2. Calculates the Conditional Stability Constant of a Metal Chelon at a Specified pH in the Presence of an Additional Complex Former

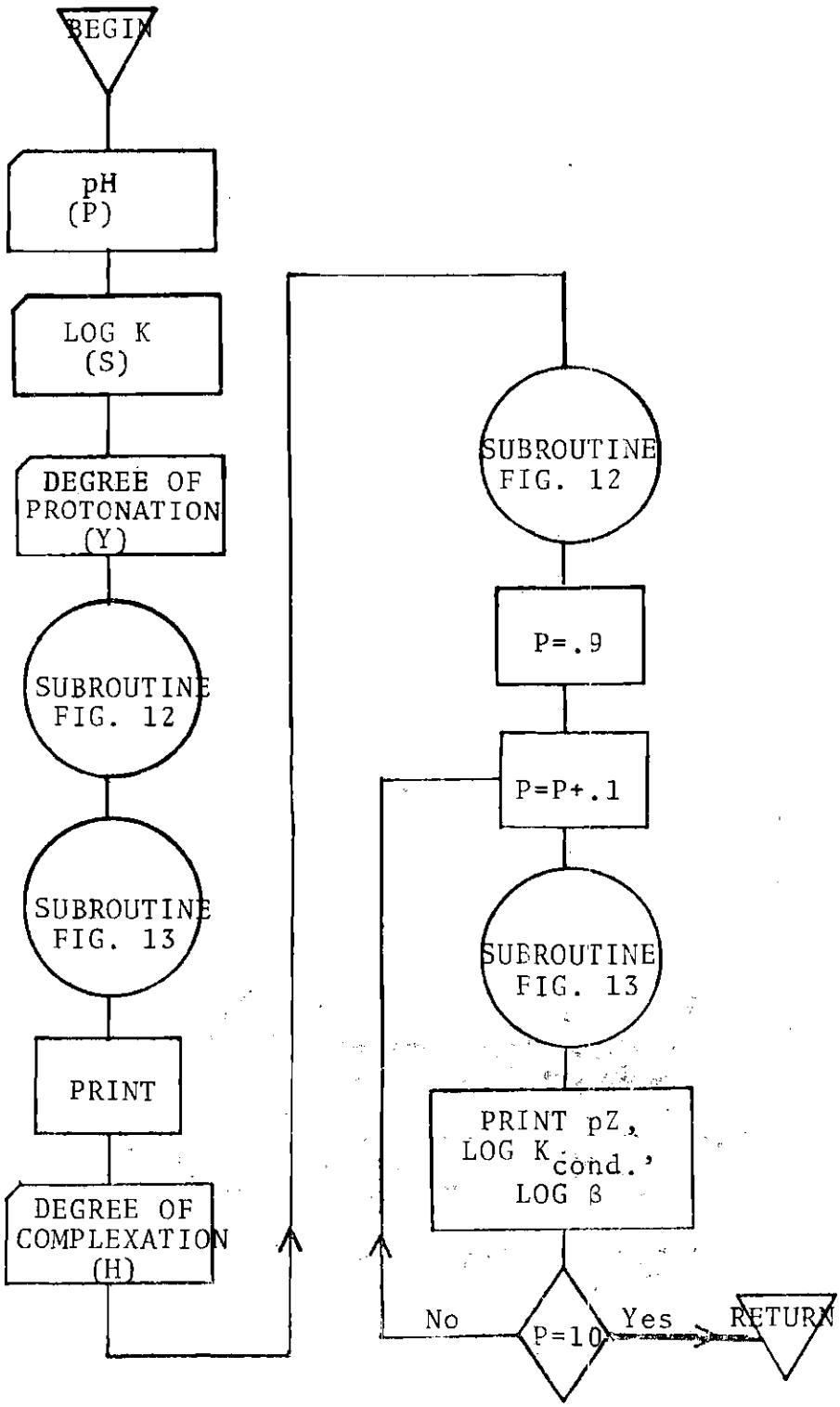

Figure 11. Flow Chart for Program 2

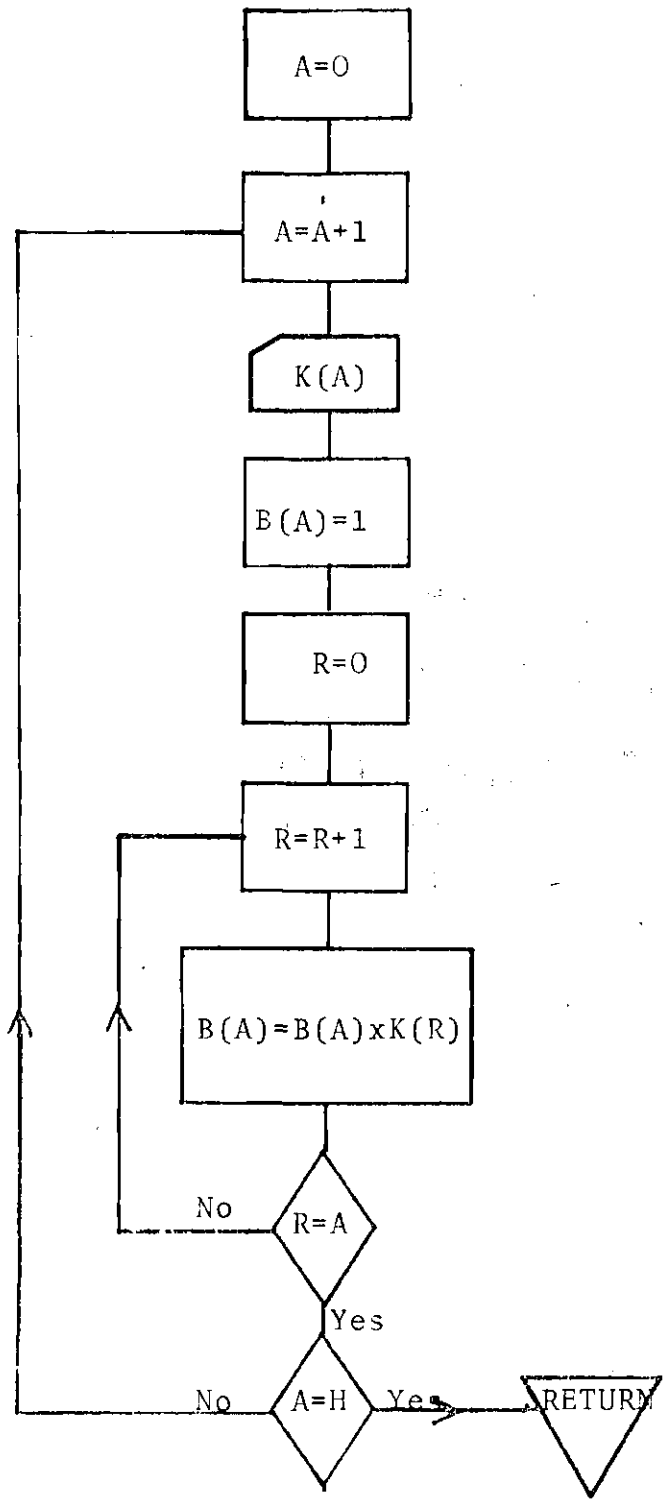

Figure 12. Flow Chart of Subroutine (Lines 100-117) of Program 2

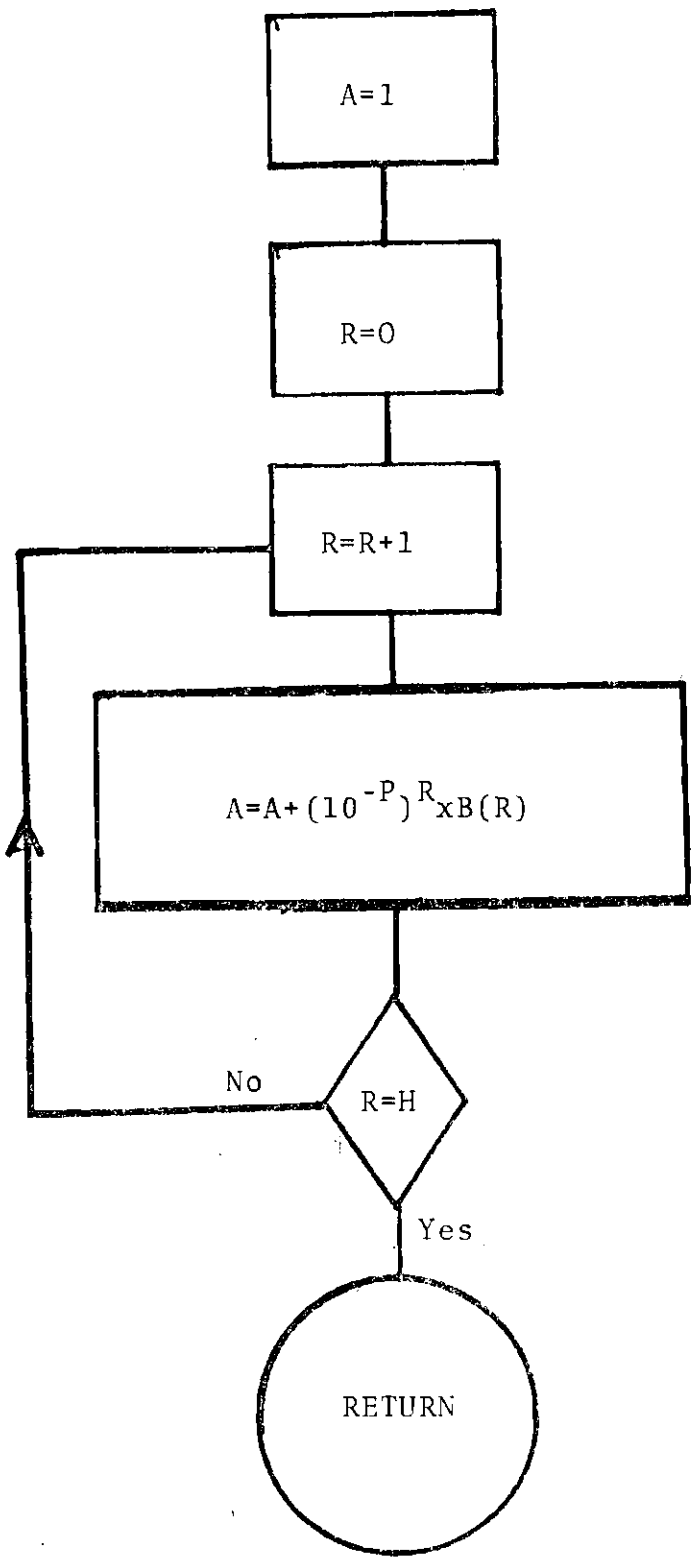

Figure 13. Flow Chart of Subroutine (Lines 120-135) of Program 2

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